DESTRUCTION OF AFLATOXIN B1 ON SORGHUM GRAIN WITH ACIDS, SALTS AND AMMONIA DERIVATIVES

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ABSTRACT — A study was conducted to determine the effect of eleven different acids, nine derivatives of ammonia and seven salts upon destruction of aflatoxin B1 on sorghum grain. Grain was adjusted to a moisture content of 25% and then treated for 72 h at 25°C. Results suggest that boric, sulphamic and sulphosalicylic acids can be used to effectively destroy aflatoxin B1 in sorghum at 5.0 N concentration. Ammonium nitrate, ammonium chloride and urea were highly effective in aflatoxin degradation at 1.5% on basis of NH4OH nitrogen weight. The use of different types of salts was efficient only in water for aflatoxin B1 destruction.

KEY WORDS: Aflatoxin B1, detoxification, acids, ammonia derivatives, salts.

INTRODUCTION

Aflatoxins are toxic secondary metabolites that can be produced on sorghum by the widely distributed fungi. Later it was found that sorghum is susceptible to aflatoxin formation by *Aspergillus* spp (Hasan & Omar, 1993). Several investigators have demonstrated that aflatoxins are acutely toxic, carcinogenic, teratogenic and mutagenic. Aflatoxin B1 is the most potent of the group and may occur naturally in grains at levels as high as mg/Kg. In our Laboratory different fungicides, herbicides, insecticides, essential oils and plant extracts have been reported as effective inhibitors in prevention of aflatoxin production by *Aspergillus* spp. (Hasan, 1993, 1994 a,b; Hasan & Mahmoud, 1993; Hasan & Omar, 1993; Hasan & Abdel-Mallek, 1994). Undoubtedly, prevention is the best method for controlling aflatoxin, however, aflatoxin that okay produced in commodiates is unavoidable by these inhibitors. Consequently, much effort has been expended in developing methods for the detoxification of aflatoxin - contaminated substances.

Detoxification of aflatoxin in feedstuffs is based on destruction of the coumarin ring to form a nontoxic metabolite. Patterson (1978) concluded that the degradation of aflatoxin B1 occurs through reductive or hydrolytic attack on the vinyl-ether double bond.

A number of reagents have been examined for their ability to reduce aflatoxin levels in contaminated agricultural commodities, included bisulfite, formaldehyde,

sodium hydroxide, sodium hypochlorite, calcium hydroxide and ammonia (Dollear, 1967, Dollear *et al.*, 1968; Goldblatt & Dollear, 1977; Bagley, 1979; Moerck *et al.*, 1980; Draughon & Childs, 1982; Norred, 1982; Hagler *et al.*, 1982 & 1983, Piva *et al.*, 1985; Mercado *et al.*, 1991). Several cooking methods used for detoxification of aflatoxin B1 in maize did not destroy more than 50% of its level (Rehana & Basappa, 1990).

Few informations on using acids, derivatives of ammonia or salts for destruction of aflatoxins are available. The aim of the present investigation was to study the ability of certain acids, ammonia derivatives and salts for destruction of aflatoxin B1 on sorghum grain at room temperature.

MATERIALS AND METHODS

50 µg of aflatoxin B1 were added to sterilized 50 g sorghum in Erlenmeyer cotton-stoppered flasks. Five concentrations of eleven different acids were prepared 0.1, 0.5, 1.0, 3.0 and 5.0 N. 2 ml of each concentration were used for treatment on sorghum grain. Nine ammonia derivatives were added separately in 0.3, 0.6, 0.9, 1.2 and 1.5% concentrations on basis of percentage NH4OH - nitrogen. Seven different salts were also used for grain treatment at two concentrations: 5 and 10%.

Sterilized distilled water was added to bring the moisture content of samples to 25% on wet basis. Samples were stored at 25°C for 72 h. All samples were returned to neutrality before extraction and purification.

Ten micrograms of aflatoxin B1 in acetone were added to 10 ml of distilled water. Two concentrations, of salts were allowed to react with aflatoxin B1 at 25°C for 72 h. Treatment and control assays were performed in triplicates.

Aflatoxin was extracted with chloroform and separated by Thin-Layer Chromatography (TLC). TLC analysis was performed on Silica Gel 60-coated plates, with a fluorescence indicator (UV 6 LC-12 W-Vilber Lourmat, France) by using chloroform: methanol (93: 3, v/v) as the developing solvent. Aflatoxin B1 was quantitated spectrophotometry at an excitation wavelength of 365 nm (Childs *et al.*, 1970) using Bausch-Lomb Spectronic 2000. Standard AF B1 was purchased from sigma (USA) and prepared in high amounts in our laboratory by fermentation with *Aspergillus flavus* IMI 89717. Aflatoxin B2a standard was prepared by addition of acetic acid or trifluoroacetic acid to aflatoxin B1. After a time of reaction, the aflatoxin B2a was detected as blue fluoresence at Rf below aflatoxin B1. The potential lower limit of visual detection for both toxins are nearly 0.4 µg.

RESULTS AND DISCUSSION

The effect of eleven different acids on aflatoxin B1 destruction in sorghum grain are illustrated in Table 1.

Most of the treatments were effective in reducing aflatoxin B1 levels at concentration 5 N. Salicylic acid appeared to be the most effective acid at 0.5 N. Treating grains for 72 h at 25°C with 3.0 N of salicylic, sulphamic and sulphosalicylic acids resulted in ≥ 90% reduction in aflatoxin B1 and an 80% reduction with anthranilic, boric and propionic acids. The rate of destruction was increased with increasing concentration of acid, up to 2 ml of 3.0 N acid per 50 g grain.

Acid	Percent destruction of aflatoxin B1				
	0.1 N	0.5 N	1.0 N	3.0 N	5.0 N
Anthranilic acid	47	53	68	80	90
Ascorbic acid *	0	0	0	24	36
Benzoic acid	17	22	42	70	90
Boric acid	0	0	0	80	100
Malic acid *	0	0	0	60	66
Oxalic acid	0	0	20	26	90
Propionic acid	38	46	48	80	90
Salicylic acid	45	66	82	90	90
Succinic acid *	18	20	24	28	40
Sulphamic acid	36	48	76	90	100
Sulphosalicylic acid	20	46	60	90	100

* - Mean aflatoxin B2a formation after treatment.

Table 1 – Effect of acids on aflatoxin B1 degradation on sorghum grain, after 72 h storage at 25°C.

Destruction of toxin was complete at 2 ml of 5.0 N boric, sulphamic and sulphosalicylic acids. Also, benzoic and oxalic induced 90% destruction in aflatoxin B1 at 5.0 N concentration with above mentioned acids (anthranilic, propionic and salicylic acids). The results obtained here-in are in accordance with data reported by Mashaly *et al.* (1983), on destruction of aflatoxins by acetic, citric and phosphoric acids. Detoxification increased with increasing concentration of acid, up to 10 ml of 0.2 N acid per 100 g cotton meal.

Thin-layer chromatography analysis, revealed a transformation of aflatoxin B1 (with ascorbic, malic and succinic acids) to a new fluorescing compound corresponding to aflatoxin B2a which referred to as hydroxydihydro-aflatoxin B1 confirmed findings of Ciegler & Paterson (1968). They found that this compound causes no bile duct hyperplasia and no death by duckling test. Also, Pohland *et al.* (1968) reported that aflatoxin B2a is formed chemically by treatment of the peanut toxin with diluted acid. Also, Hafez & Megalla (1982) convinced that acidity was responsible for the transformation of aflatoxin B1 to B2a and the conversion proceed non-enzymatically.

The effect of nine ammonia derivatives on aflatoxin B1 level in sorghum grain are illustrated in Table 2.

Ammonia derivatives	Percent destruction of aflatoxin B1				
	0.3%	0.6%	0.9%	1.2%	1.5%
Ammonium acetate	37	39	42	50	55
Ammonium chloride	45	50	62	80	93
Ammonium molybdate tetrahydrate	25	39	45	65	72
Ammonium nitrate	57	75	85	87	96
Ammonium oxalate	38	45	47	50	55
Ammonium phosphate	50	53	60	63	65
Ammonium sulphate	45	50	56	64	67
Ammonium tartarate	25	37	45	60	70
Urea	26	47	50	75	93

Table 2: Aflatoxin destruction on sorghum grain by treatment with ammonia derivatives during 72h at 25°C.

Most of ammonia treatments were effective in reducing aflatoxin B1 level especially when they are performed at concentrations as low as 1.5%. Ammonium nitrate appeared to be more effective at lower concentrations. Treating sorghum for 72 h with 0.6% ammonium nitrate resulted in a 75% reduction in aflatoxin and an 96% reduction at 1.5% level. Treating this grain with 1.5% ammonium chloride or urea resulted in a decrease in aflatoxin B1 of 93%. Ammonium sulphate, ammonium tartarate, ammonium phosphate or ammonium molybdatetetrahydrate reduced aflatoxin B1 by 60-65% at 1.2% concentration. While treatment with 1.2% ammonium acetate or ammonium oxalate resulted in a decreased in aflatoxin of 50%.

The mechanism of aflatoxin degradation by ammoniation has been proposed by Lee *et al.* (1974), Kiermeier & Ruffer (1974) and Cucullu *et al.* (1976). Purportedly, ammonia reacts with aflatoxin B1 at the lactone ring to form the ammonium salt derivative. Loss of ammonia results in the formation of the keto acid which is subsequently decarboxylated to form aflatoxin D1. An alternate mechanism leads to the formation of furofurophenal. Aflatoxin D1 did not notice on TLC after ammonia derivatives treatment in our study.

Results of the degradation of aflatoxin after salts treatment in distilled water or sorghum grains are presented in Table 3.

Salts	Water		Grain	
	5%	10%	5%	10%
Aluminium chloride	55	84	30	50
Barium chloride	30	70	10	40
Calcium chloride	58	85	30	53
Potassium bromide	50	82	35	55
Potassium cromate	40	79	25	45
Sodium borate	47	80	40	59
Sodium fluoride	60	85	40	60

Table 3 — Effect of salts on aflatoxin B1 degradation in water and sorghum grain, after 72h storage at 25°C.

All tested salts appear to be more efficient for aflatoxin destruction in water than on sorghum grains. Addition of 10% of these salts separately in distilled water reduced aflatoxin by about 70-85%. In sorghum grains the salts reduced aflatoxin by about 40-60% only. The most efficient salt was sodium fluoride followed by calcium chloride, aluminum chloride, potassium bromide and sodium borate.

CONCLUSION

As expected, the concentration of substance needed will depend on the amount of aflatoxin in the grain, because the detoxification process is a chemical reaction (Patterson, 1978). Therefore, the results of this investigation demonstrate that boric, sulphamic and sulphosalicylic acids can be used for eliminating 1000 µg aflatoxin B1 in kg sorghum at concentration of 5.0 N. Also, ammonium nitrate, ammonium chloride and urea were also efficient for aflatoxin destruction. Use of salts is not applicable to aflatoxin destruction on grains, but efficient in water.

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